

Susceptibility of *Bordetella pertussis* to Cephalosporin Derivatives and Imipenem

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The in vitro activities of 16 cephalosporin derivatives and imipenem against 60 strains of *Bordetella pertussis* were examined. Cefoperazone, imipenem, and ceftriaxone were the most active, with MICs that inhibited 90% of the strains of 0.006, 0.05, and 0.1 µg/ml, respectively. Cephalixin, cephradine, and cefsulodin were the least active, with MICs of 25 to 125 µg/ml. The remainder of the agents demonstrated intermediate activity.

The relative resistance of *Bordetella pertussis* over other respiratory flora to cephalixin has been made use of for many years in the formulation of selective isolation media (3, 5). However, whether cephalixin is the ideal cephalosporin derivative for this purpose merits reevaluation in view of the recent proliferation of these compounds. Also, since several organisms resistant to the earlier cephalosporin derivatives are susceptible to the new ones (1), a systematic examination of the in vitro activity of the new cephalosporin derivatives against *B. pertussis* could reveal potentially useful therapeutic agents.

Sixty separate clinical isolates of *B. pertussis* were tested against 16 cephalosporin derivatives and imipenem. Most strains had been passaged only a few times between isolation or lyophilization and testing. They were grown on Bordet-Gengou medium for 3 days before inoculation into Stainer-Scholte broth (4). The cultures were incubated at 37°C for 1 day in a water bath with shaking to produce a suspension with an absorbance of 0.6 at 560 nm on a spectronic 20 spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.). This was equivalent to a density of 10^9 cells per ml as determined by viable count. The suspension was further diluted 1:100 with Stainer-Scholte broth so that each prong of the replicator head delivered a final inoculum of 5×10^4 cells. Susceptibility tests for the cephalosporin derivatives and imipenem were performed on Bordet-Gengou blood agar.

Cefaclor, cephalothin, moxalactam, and cefamandole (Eli Lilly & Co., Toronto, Ontario) as well as cefoxitin (Frosst, Montreal, Quebec), cefsulodin (Abbott Laboratories, North Chicago, Ill.), ceftizoxime (Smith Kline & French Laboratories, Philadelphia, Pa.), ceftriaxone (Hoffmann-La Roche Ltd., Quebec), cefuroxime (Glaxo, Inc., Weston, Ontario), and cephradine (Squibb Canada Inc., Montreal, Quebec) were dissolved in sterile, distilled water. Cefazolin (Smith Kline & French Laboratories, Mississauga, Ontario), cefotaxime (Roussel, Montreal, Quebec), cephalixin (Eli Lilly), and cephaloridine (Glaxo) were dissolved in 0.02 M phosphate buffer (pH 7.2). Cefoperazone (Pfizer Co. Ltd., Pointe-Claire, Quebec) was dissolved in 0.025 M phosphate buffer (pH 5.5). Ceftazidime (Glaxo) was dissolved in 0.01% Na₂CO₃, and imipenem (Merck Frost Canada Inc., Pointe-Claire, Quebec) was dissolved in 0.01 M phosphate buffer (pH 7.2).

The agar dilution method of susceptibility testing was used. *B. pertussis* strains were inoculated onto Bordet-

Gengou blood agar plates, using a multiple-replicator apparatus. Plates were used within 5 days of preparation except those containing unstable antibiotics, which were prepared fresh. They were incubated at 37°C in the dark for 42 to 45 h. Susceptibility was defined as the absence of visible growth at the point of inoculation. All strains were tested in duplicate. Sterility controls, drug-free solvent controls, growth controls, and control strains of *Haemophilus influenzae* and *Pseudomonas aeruginosa* with known MICs on Bordet-Gengou medium were included in the assessments. MICs that inhibited 50 and 90% of the strains (MIC₅₀ and MIC₉₀) were calculated by the Reed and Muench method (2).

The susceptibility of *B. pertussis* to the drugs tested, expressed in terms of inhibitory range, MIC₅₀, and MIC₉₀, are arranged in Table 1. Cephalixin and the closely related cephradine were the least active (MICs of 125 and 100 µg/ml), and cefsulodin, the narrow-spectrum new cephalosporin derivative, was also only weakly active (MIC of 50.0 µg/ml). Cefoxitin, cefamandole, cefuroxime, cephalothin, ceftizoxime, cefazolin, cefaclor, and cephaloridine had MICs in the intermediately active range of 1 to 25 µg/ml. Only imipenem and the new cephalosporin derivatives cefotaxime, ceftazidime, ceftriaxone, and cefoperazone were active at concentrations below 1.0 µg/ml, and of these, cefoperazone appeared to be the most inhibitory (MIC of 0.012 µg/ml).

TABLE 1. Susceptibility of *B. pertussis* to cephalosporin derivatives and imipenem

Antibiotic	MIC (µg/ml)		
	Range	50%	90%
Cephalixin	31-125	64	109
Cephradine	50-100		
Cefsulodin	25-50		
Cephaloridine	3-25	7.9	20.7
Cefaclor	12-25		
Cefazolin	6-12	6.3	10.9
Ceftizoxime	6-12	7.1	11.2
Cephalothin	0.6-10	5.6	8.9
Cefuroxime	1.5-6.0	2.8	5.3
Cefamandole	1-2	1.6	2.3
Cefoxitin	1-2	1.1	2.1
Moxalactam	0.5-1		
Imipenem	0.08-0.6	0.03	0.05
Cefotaxime	0.16-0.6	0.2	0.3
Ceftazidime	0.16-0.3	0.2	0.29
Ceftriaxone	0.06-0.12	0.05	0.1
Cefoperazone	0.003-0.012	0.003	0.006

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During the screening of the early cephalosporin derivatives against *B. pertussis* for potentially useful therapeutic activity, the relative resistance of the organism was noted. This fact was made use of in the development of selective enrichment and isolation media (3, 5). There are no records of the activity of later cephalosporin antibiotics in this regard. Also, in view of the expanded spectrums and more intense activities of the new cephalosporin derivatives and imipenem, a reexamination of the susceptibility status of the organism to these agents appears overdue.

The present study documents the susceptibility of 60 strains of *B. pertussis* to the early, late, and new agents. By and large, the isolates demonstrated either outright resistance or only limited susceptibility to the so-called first-generation drugs. Not unexpectedly, cephalexin and the closely related cephradine showed the weakest activity. The new cephalosporin derivative, cefsulodin, with its somewhat narrow spectrum, also demonstrated a high MIC. Since *P. aeruginosa*, which is susceptible to cefsulodin, is one of the few bacteria to regularly (though infrequently) break through the cephalexin barrier in standard isolation media, consideration might be given to the use of cefsulodin as an alternative

selective agent. A number of agents showed levels of activity of limited use to bacteriologists and clinicians. But some of the newer derivatives exhibited inhibitory activities in the therapeutic range at levels comparable to those encountered against enteric gram-negative organisms or *H. influenzae*. In circumstances in which a parenteral agent is indicated for the antibiotic management of pertussis, drugs such as ceftriaxone and cefoperazone might merit some consideration.

LITERATURE CITED

1. Neu, H. C. 1982. The new beta-lactamase-stable cephalosporins. *Ann. Int. Med.* **97**:408-419.
2. Reed, L. J., and H. Muench. 1938. A simple method for estimating fifty percent endpoints. *Am. J. Hyg.* **27**:493-497.
3. Regan, J., and F. Lowe. 1977. Enrichment medium for the isolation of *Bordetella*. *J. Clin. Microbiol.* **6**:303-309.
4. Stainer, D. W., and M. J. Scholte. 1970. A simple chemically-defined medium for the production of phase I *Bordetella pertussis*. *J. Gen. Microbiol.* **63**:211-220.
5. Sutcliffe, E. M., and J. D. Abbott. 1972. Selective medium for the isolation of *Bordetella pertussis* and *Parapertussis*. *J. Clin. Pathol.* **6**:732-733.